RNAi-Mediated Inhibition of a Natural Anticoagulant for the Treatment of Hemophilia

OTS 2012—Session VI: RNAi/ASO Preclinical Studies

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October 30, 2012
Hemophilia

- X-linked, recessive bleeding disorders caused by deficiency of functional clotting factors VIII and IX

<table>
<thead>
<tr>
<th></th>
<th>Hemophilia A</th>
<th>Hemophilia B</th>
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<tbody>
<tr>
<td>Percent of Hemophilic Population</td>
<td>80%</td>
<td>20%</td>
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<tr>
<td>Deficient Clotting Factor</td>
<td>Factor VIII (FVIII)</td>
<td>Factor IX (FIX)</td>
</tr>
<tr>
<td>Approximate Incidence¹</td>
<td>1 in 5,000 male births</td>
<td>1 in 20,000 male births</td>
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<tr>
<td></td>
<td>400 new cases each year</td>
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<tr>
<td>Reported number of Hemophilia Patients in US/EU²</td>
<td>~41,000</td>
<td>~9,600</td>
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</tbody>
</table>

2. WFH Global survey 2009
Medical Impact

- Typically do not suffer from hemorrhage from minor cuts or abrasions due to normal platelet count and function
- Availability (in developed nations) of safe plasma-derived and recombinant factor has lead to near normal life expectancies; however,
- Recurrent joint and soft-tissue bleeds leads to disabling arthropathy
- Potential life-threatening complications associated with surgery or acute trauma
- Development of inhibitors to replacement factors renders patients more risky and costly to treat

Current Management and Unmet Need

**Current Management**

- Treated with replacement factors, either as “on-demand” or prophylaxis
- Prophylaxis is considered standard of care in developed countries for severe hemophilia, particularly for pediatric population
  - Typically 2-3 times per week IV infusion (w/target 1% trough level)
  - Prevention of bleeds protects joint function
- Patients with inhibitors are most challenging to treat
  - Immune tolerance induction (ITI) therapy may be attempted to clear inhibitor
  - Generally treated with “bypass agents” (rFVIIa, APCCs)
    - Prophylaxis not feasible due to short half-life and cost of bypass agents

**Unmet Need**

- Treatment of inhibitor patients is area of greatest unmet need
  - Feasible prophylaxis option is needed to prevent arthropathy
  - ITI very demanding, costly protocol and not always successful
  - Bypass agents very costly and have short half-life
- Prophylaxis is costly and inconvenient due to need for frequent IV infusion
  - Frequent IV access can require ports, particularly in children, sometimes resulting in complications (e.g. infection)
  - Target factor trough levels of 1% are not sufficient to prevent all bleeds
  - Cost and frequent IV infusion protocol is an impediment for adoption of prophylaxis by some adults
- Effective therapies for the developing world
Therapeutic Hypothesis
Rebalancing the Hemostatic System

Intrinsic system
Hemophilia B
FVII \rightarrow FVIIa

Hemophilia A
FX \rightarrow FIXa

Extrinsic system
FX \rightarrow FVIIa

FVII \rightarrow FVIIa

FVa \rightarrow FVa

FV \rightarrow FVa

Prothrombin \rightarrow Thrombin

Thrombin \rightarrow Fibrinogen

Fibrinogen \rightarrow Fibrin

Blood clot
Prothrombotic Factors in Hemophilia

Co-inheritance of Prothrombotic factors and hemophilia
- Prothrombotic factors include Factor $V_{\text{Leiden}}$ (FVL), Prothrombin G20210A, Protein C deficiency, Protein S deficiency
- Number of reports of hemophilia patients with co-inheritance of prothrombotic factors with milder disease, e.g. reduced bleeding episodes, arthropathy and FVIII requirements

Symptom-free survival in children with severe hemophilia A (HA) with and without additional prothrombotic risk factors

Median age of first symptomatic bleed in severe HA:
- 1.6 years in children with prothrombotic factors:
  - FVL (6)
  - Prothrombin (3)
  - Protein C (type I) deficiency (1)
- 0.9 years in non-carriers

Impact of FVL On Severe Hemophilia A Phenotype

Table 2. Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>No thrombophilia</th>
<th>With thrombophilia</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first bleeding</td>
<td>0.9 [0.1-4.0]</td>
<td>1.5 [0.5-7.1]</td>
<td>0.009</td>
</tr>
<tr>
<td>(median/range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Therapy given: number [%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>on demand</td>
<td>58 [63.0]</td>
<td>8 [53.3]</td>
<td>0.67</td>
</tr>
<tr>
<td>prophylaxis</td>
<td>34 [37.0]</td>
<td>7 [46.7]</td>
<td></td>
</tr>
<tr>
<td>Start of prophylactic regimen: Median/range values (years)</td>
<td>1.3 [0.1-6.7]</td>
<td>1.9 [0.8-7.0]</td>
<td>0.44</td>
</tr>
<tr>
<td>Factor concentrates used [%]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pdFVIII</td>
<td>27.3</td>
<td>33.3</td>
<td>0.33</td>
</tr>
<tr>
<td>rFVIII</td>
<td>48.5</td>
<td>55.5</td>
<td></td>
</tr>
<tr>
<td>WFVIII</td>
<td>24.2</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>Annual bleeding frequency</td>
<td>6 [0.3-30]</td>
<td>1.8 [0.7]</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Kurnik et al., Hematologica; 92: 982-985 (2007)

Lee et al., Thromb Haemost; 83: 387-391 (2000)
## Natural Anticoagulant Factors

<table>
<thead>
<tr>
<th>Anticoagulant</th>
<th>Mechanism</th>
<th>Genetics</th>
<th>Expression</th>
</tr>
</thead>
</table>
| Protein C    | • Inactivates FVa and FVIIIa  
               • Cytoprotective functions; gene expression, anti-inflammatory effects, antiapoptotic effects and protecting endothelial barrier | • Heterozygous PC deficiency associated with increased risk for venous thromboembolism (VTE) | Hepatocytes |
| Antithrombin  | • Direct stoichiometric inhibitor of thrombin and FXa | • Heterozygous AT deficiency associated with increased risk for VTE | Hepatocytes |
| Protein S    | • Cofactor of APC for inactivation of FVa and FVIIIa | • Heterozygous PS deficiency associated with increased risk for VTE | Mainly hepatocytes and vascular endothelium |
| TFPI         | • Binds to FXa, and forms inhibitory quaternary complex with FVIIa-TF | • None described | Mainly vascular endothelium |
Antithrombin is hepatocyte-expressed serpin

- Encoded by SERPINC1 on Ch1
- Abundant plasma glycoprotein (58 kDa)

Anticoagulant function

- Major role to inhibit thrombin and FXa
- Inhibits other activated factors VIIa, IXa, XIa, and XIIa to lesser degree
- Forms inhibitory stochiometric complex with serine protease
- Activity greatly enhanced by heparin cofactor
  » Results in ternary complex with thrombin, enhances thrombin inhibition rate 2000-fold
  » Induces conformational change, enhances FXa inhibition rate 500-1000-fold

Human Genetics of Antithrombin Deficiency

Incidence
- Estimated between 1 in 500 and 1 in 5000
- AT deficiency classified as Type I (quantitative) or Type II (qualitative) defects
  » Type I and Type II defects make up 12% and 88% of cases of AT deficiency, respectively
  » However, Type I defects represent up to 80% of symptomatic cases of AT deficiency

Phenotype
- Homozygous deficiency is almost always fatal *in utero*
- Heterozygous deficiency is associated with 40-60% of normal AT activity levels
- AT deficiency is associated with the highest VTE risk among inherited thrombophilias
- Risk of VTE depends heavily on personal history, family history, and subtype of AT deficiency
  » Approximately 40-60% of incidence attributable to other transient risk factors (e.g. surgery, pregnancy, puerperium, immobilization, oral contraceptives)
  » Asymptomatic patients have ~1.7%/year risk of VTE
  » Patients with incidence of VTE not on long-term anticoagulation have 10-17%/year risk of recurrence
  » Patients with incidence of VTE on long-term anticoagulation have 2.7%/year risk of recurrence

Treatment
- Asymptomatic patients are not on long-term thromboprophylaxis
- Patients with incidence of VTE are considered for long-term thromboprophylaxis
- Standard thromboprophylaxis for surgery and immobility

Proof of Concept in Mouse Model of Hemophilia

**Increased Thrombin Generation**

![Thrombin Generation Graph](image)

**Reduced Blood Loss**

![Blood Loss Graph](image)

<table>
<thead>
<tr>
<th></th>
<th>WT (n=15)</th>
<th>AT+/− (n=15)</th>
<th>FVIII−/− (n=15)</th>
<th>FVIII−/−/AT+/− (n=15)</th>
<th>FVIII+/− (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lag time (min)</td>
<td>2.3 ± 0.9</td>
<td>2.8 ± 1.1</td>
<td>1.9 ± 4.0</td>
<td>1.2 ± 0.7</td>
<td>2.1 ± 2.1</td>
</tr>
<tr>
<td>Thrombin peak (nM)</td>
<td>45 ± 22</td>
<td>58 ± 22</td>
<td>10 ± 11*</td>
<td>29 ± 17</td>
<td>37 ± 26</td>
</tr>
<tr>
<td>ETP (nM)</td>
<td>600 ± 497</td>
<td>831 ± 291</td>
<td>94 ± 237*</td>
<td>303 ± 258</td>
<td>390 ± 291</td>
</tr>
<tr>
<td>Slope (nM/min)</td>
<td>10.5 ± 6.7</td>
<td>15.1 ± 4.1</td>
<td>2.0 ± 2.1*</td>
<td>4.1 ± 3.1*</td>
<td>8.3 ± 6.1</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Intergroup differences were evaluated by ANOVA followed by Bonferroni’s posthoc test. * = p < 0.01 vs. WT mice. $\dagger$ = p < 0.01 vs. FVIII+/− mice.

Proof of Concept in Human Plasma Model of Hemophilia

**Thrombin Generation**
in FIX- and AT-Depleted Human Plasma

- 0% FIX, 100% AT
- 0% FIX, 75% AT
- 0% FIX, 50% AT
- 0% FIX, 25% AT
- 0% FIX, 10% AT
- 0% FIX, 5% AT
- 0% FIX, 0% AT
- 100% FIX, 100% AT

**Peak Thrombin**
in FIX- and AT-Depleted Human Plasma

- 100% FIX, 100% AT

*In collaboration with Haematologic Technologies, Inc.*
siRNA Conjugate Approach
For Targeted Delivery to Hepatocytes

**GalNAc-siRNA**
- Trivalent GalNAc carbohydrate cluster has high affinity (nM) for ASGPR
- GalNAc ligand conjugated to chemically-modified, AT-targeting siRNA
- Administered subcutaneously (SC)

**ASGPR**
- Highly expressed in hepatocytes ➔ 0.5-1 million copies/cell
- Clears serum glycoproteins via clathrin-mediated endocytosis
- High rate of uptake
- Recycling time ~15 minutes
- Conserved across species
Administration of ALN-AT3
Potent and Durable Suppression of Antithrombin in Mice

Dose Response
- Single subcutaneous dose
- N = 5, 72 hour time point
- AT protein measured in serum
- AT mRNA measured in liver

Duration
- Single subcutaneous dose
- N = 5
- AT protein measured in serum

![Graph showing dose response and duration of ALN-AT3 suppression of antithrombin in mice.](image-url)
Maintenance of AT Levels
Subcutaneous Weekly Injections

Weekly dosing results in sustained suppression of AT levels, with minimal deviations from steady-state levels
Weekly and 3X Weekly Dosing Yields Similar Steady-State Silencing

- **0.75 mg/kg** cumulative weekly
- **1.5 mg/kg** cumulative weekly
- **3 mg/kg** cumulative weekly

**Relative Serum AT (PBS, Pre-dose = 1)**

- **0.75 mg/kg, q1w**
- **0.25 mg/kg, t.i.w.**

- **1.5 mg/kg, q1w**
- **0.5 mg/kg, t.i.w.**

- **3 mg/kg, q1w**
- **1 mg/kg, t.i.w.**

**Day**

0 4 8 12 16 20 24 28 32
Weekly and Every Other Weekly Dosing Yields Similar Steady-State Silencing

0.25 mg/kg cumulative weekly

0.5 mg/kg cumulative weekly

Relative AT Protein Level (Pre-dose = 1)

Day

0 10 20 30 40 50 60

0 0.2 0.4 0.6 0.8 1.0 1.2 1.4

0 10 20 30 40 50 60

0 0.2 0.4 0.6 0.8 1.0 1.2 1.4

0.25 mg/kg q1w 0.5 mg/kg q2w

0.5 mg/kg q1w 1 mg/kg q2w
ALN-AT3 Mouse PK Data

- ALN-AT3 persisted in liver to last time-point collected
- Exposure after SC administration was much higher after IV administration
- % of dose in liver after SC administration at Tmax was 53 %
Hypothesis: AT knock-down should increase thrombin generation in hemophilic mice

- \( N = 3 \), hemophilia B (FIX \(^{+/−}\) ) mice or wild-type mice
- Single subcutaneous injection at 30 mg/kg
- 72 hour time point
- Blood collected in sodium citrate with CTI
- TGA using Calibrated Automated Thrombinscope (CAT)

In collaboration with Dr. Claude Negrier

Thrombin Generation in HB Mice

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>WT Control</th>
<th>HB Control</th>
<th>ALN-AT3SC treated #1</th>
<th>ALN-AT3SC treated #2</th>
<th>ALN-AT3SC treated #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>40</td>
<td>10</td>
<td>15</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>25</td>
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<td>40</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>20</td>
<td>40</td>
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Normalization of ETP in HB Mice

<table>
<thead>
<tr>
<th>Thrombin Generation AUC (nM min)</th>
</tr>
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<tbody>
<tr>
<td>WT Control</td>
</tr>
<tr>
<td>HB Control</td>
</tr>
<tr>
<td>ALN-AT3SC Treated</td>
</tr>
</tbody>
</table>

In collaboration with Dr. Claude Negrier
ALN-AT3 Activity in Nonhuman Primates

Experimental Design
• Wild-type cynomolgus monkeys, N = 3
• Single SC injection (1 mL/kg)

Time Course

Day 15

Relative Serum AT
(Pre-dose = 1)

0.0
0.2
0.4
0.6
0.8
1.0
1.2

Day

10 mg/kg
3.0 mg/kg
1.0 mg/kg

ALN-AT3SC (mg/kg)

0.0
0.2
0.4
0.6
0.8
1.0
1.2

1.0
3.0
10
ALN-AT3 treatment results in potent inhibition of AT in mice and NHPs

Given long duration of action, twice monthly SC dosing is likely possible

Proof-of-concept achieved in experimental settings with AT reduction

- Increased thrombin generation in human factor-depleted plasma
- Normalization of thrombin generation in hemophilia mouse

Hemostatic rebalancing approach utilizing ALN-AT3 represents a potentially new prophylaxis therapy option in persons with hemophilia and rare bleeding disorders
Acknowledgements

Alnylam Pharmaceuticals
  • A. Sehgal
  • J. Brodsky
  • T. Racie
  • J. Qin
  • S. Barros
  • J. Hettinger
  • D. Foster
  • S. Milstein
  • K. Charisse
  • S. Kuchimanchi
  • M. Maier
  • R. Kallanthottathil
  • B. Bettencourt
  • A. Simon

Edouard Herriot University Hospital, Lyon, France
  • Y. Dargaud
  • C. Negrier
Thank You